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Cytotaxonomic Study of 12 Species in the Polypodiaceae from Southern China

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ABSTRACT.—Chromosome numbers and reproductive biologies of 12 fern species from eight genera in the Polypodiaceae, *(Colysis, Lepidogrammitis, Lepisorus, Microsorium, Neolepisorus, Phymatopteris, Phymatosorus,* and *Pyrrosia)* from the southern region of China (Yunnan, Guangxi and Hainan) were investigated. The base number is $x=36$ in these genera of Polypodiaceae. The chromosome numbers for four species: $2n=72$ ($2x$) in *Lepidogrammitis drymoglossoides*, $2n=72$ ($2x$) in *Neolepisorus ovatus*, $2n=72$ ($2x$) in *Phymatopteris rhynchophylla*, and $2n=72$ ($2x$) in *Phymatosorus hainanensis* are here reported for the first time. Four records: $2n=72$ ($2x$) in *Colysis hemionitidea*, $2n=72$ ($2x$) in *Lepisorus thunbergianus*, $2n=108$ ($3x$) in *Phymatopteris crenatopinnata*, and $2n=108$ ($3x$) in *Phymatosorus cuspidatus* are new cytotypes. *Lepisorus thunbergianus* $2n=72$ ($2x$) has the base number of $x=36$, diverging from those cited in previous reports ($x=25$, 38, 50, and 51). The reproductive type in *P. crenatopinnata* and *P. cuspidatus* is apogamous, whereas in the other species it is of the sexual reproductive type.

KEY WORDS.—China, Chromosome number, Cytotaxonomy, Polypodiaceae, Reproductive mode

The Polypodiaceae is one of the most diverse groups of extant ferns (Schneider *et al.*, 2004). They are not only a lineage of derived ferns that has a high number of species, but they also display a vast range of morphological variation (Kreier *et al.*, 2008). Ching believed that China (especially SW China and the Himalayas) was the center of origin of Polypodiaceae, with about half the number of the total species of Polypodiaceae occurring there (Ching, 1979; Lin, 2000).

Cytological and reproductive studies may provide very useful information for phylogenetic and evolutionary studies of various groups of ferns. Data on chromosome numbers, base numbers, karyotypes, aneuploids, polyploidy levels, and reproductive modes are useful for understanding of the origin,

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evolution and speciation of the ferns (Manton, 1950; Wang, 1984; Wu, 1984; Wang *et al.*, 2007). Data on chromosomes are also important for discriminating species and species complexes.

Previous cytological studies have recorded chromosome numbers in less than 60 species of Polypodiaceae in South China (Yunnan, Zhejiang, Hainan, and Fujian) (Wang, 1984; Weng, 1990; Kato *et al.*, 1992; Cheng, 1992; Kato and Nakato, 1999; Nakato *et al.*, 1995; Lin *et al.*, 1996, 2002). This number accounts for only 23% of the Chinese Polypodiaceous ferns. Data on chromosomes of some genera/species in Polypodiaceae have been reported, such as $2n=72$ ($2x$) in *Lepidogrammitis rostrata*, *Phymatopteris oxyloba* and *Lepisorus macrosphaerus*; $2n=108$ ($3x$) in *Phymatopteris trisecta*; and $2n=70$ ($2x$) in *Lepisorus bicolor* and so on (Wang *et al.*, 2007; Wang and Lu, 2008; Lu and Wang, 2008).

Cytological research on these ferns has been severely lacking, because of difficulties in manipulating root tips due to their small size, inaccessibility of the species, and the large number of chromosomes. Cytological studies of the southern China Polypodiaceous ferns can contribute to our understanding of the origin and evolution of the Polypodiaceae. Accordingly, we chose 12 species in eight genera of Polypodiaceae as materials, following the alphabet order. Chromosome counts had already been performed for eight of the species used in this study: $n=36$ in *Colysis elliptica* (Tsai and Shieh, 1983; Weng, 1990), $2n=144$ ($4x$) in *C. hemionitidea* (Mehra, 1961), $2n=72$ ($2x$) in *C. pothifolia* (Tsai and Shieh, 1983; Kato *et al.*, 1992), $2n=50$, 51, 75, 76, 100, 101, 102 and 103 in *Lepisorus thunbergianus* (Takamiya, 1996), $n=36$, 37 in *Microsorium fortunei* (Roy and Holttum, 1965; Mitui, 1973), $2n=72$ ($2x$) in *Phymatopteris crenatopinnata* (Panigrahi and Patnaik, 1961), $2n=74$ ($2x$) in *Phymatosorus cuspidatus* (Kato and Nakato, 1999), and $2n=72$ ($2x$) in *Pyrrosia lingua* (Takei, 1969, 1983a). The aim of this study is to provide new cytological information on the classification and phylogenetics of Polypodiaceous ferns.

Materials and Methods

Living plants (Table 1, Fig. 1) were collected from the field in Yunnan, Guangxi and Hainan provinces, southern China, and cultivated in the lab. Voucher specimens were deposited in the Herbarium of Yunnan University (PYU).

For the examination of mitotic chromosomes, root tips were pretreated in 0.002 mol/L 8-hydroxyquinoline solution for 3–6h before being fixed in Carnoy's solution (95% ethanol: glacial acetic acid=3:1) for 12–24 h; then they were hydrolyzed in 1 mol/L HCl at 60°C for 10–15 min. After washing 3–4 times to eliminate residual hydrochloric acid, materials were stained in 2% aceto-orcein and squashed by the usual method (Manton, 1950). The "second squashed" method (Wang and Zhang, 1981) was used when chromosomes occurred in different planes. Mitotic cells were examined and photographed by using an Olympus BX51-DP70 photomicroscope. More than 30 chromosome micrographs were observed in each individual.

In the higher leptosporangiate ferns, there are two reproductive types: the sexual reproductive type with 64 spores in a sporangium, and the apogamous

TABLE 1. Taxa studied of Polypodiaceae, their collection information and chromosome data.

Taxon	Chromosome number	Spore N.*	Locality	Elevation (m)	Voucher	Figs
Colysis elliptica (Thunb.) Ching	2n=72	64	Mulun, Guangxi	350	XC Deng *et al.* 31786 (PYU)	2A, 3A
Colysis hemionitidea (Wall. ex Mett.) C. Presl	2n=72	64	Guilin, Guangxi	210	RX Wang *et al.* 017 (PYU)	2B, 3B
Colysis pothifolia (D. Don.) C. Presl	2n=72	64	Guilin, Guangxi	280	RX Wang *et al.* 010 (PYU)	2C, 3C
Lepidogrammitis drymoglossoides (Bak.) Ching	2n=72	64	Bama, Guangxi	380	RX Wang *et al.* 015 (PYU)	2D, 3D
Lepisorus thunbergianus (Kaulf.) Ching	2n=72	64	Huanjiang, Guangxi	450	XC Deng *et al.* 31758 (PYU)	2E, 3E
Microsorium fortunei (T. Moore) Ching	2n=72	64	Guilin, Guangxi	180	XC Deng *et al.* 31790 (PYU)	2F, 3F
Neolepisorus ovatus (Bedd.) Ching	2n=72	64	Guilin, Guangxi	160	RX Wang *et al.* 014 (PYU)	2G, 3G
Phymatopteris crenatopinnata (C. B. Clarke) Pichi-Serm.	2n=108	32	Weixi, Yunnan	2300	RX Wang *et al.* 009 (PYU)	2H, 3H
Phymatopteris rhynchophylla (Hook.) Pichi-Serm.	2n=72	64	Pingbian, Yunnan	1700	RX Wang *et al.* 008 (PYU)	2I, 3I
Phymatosorus cuspidatus (D. Don.) Pichi-Serm.	2n=108	32	Bama, Guangxi	520	RX Wang *et al.* 016 (PYU)	2J, 3J
Phymatosorus hainanensis (Noot.) S. G. Lu	2n=72	64	Mt. Wuzhishan, Hainan	250	SG Lu 4001 (PYU)	2K, 3K
Pyrrosia lingua (Thunb.) Farwell	2n=72	64	Pingbian, Yunnan	1600	XC Deng *et al.* 31797 (PYU)	2L, 3L

* Number of spores per sporangium.

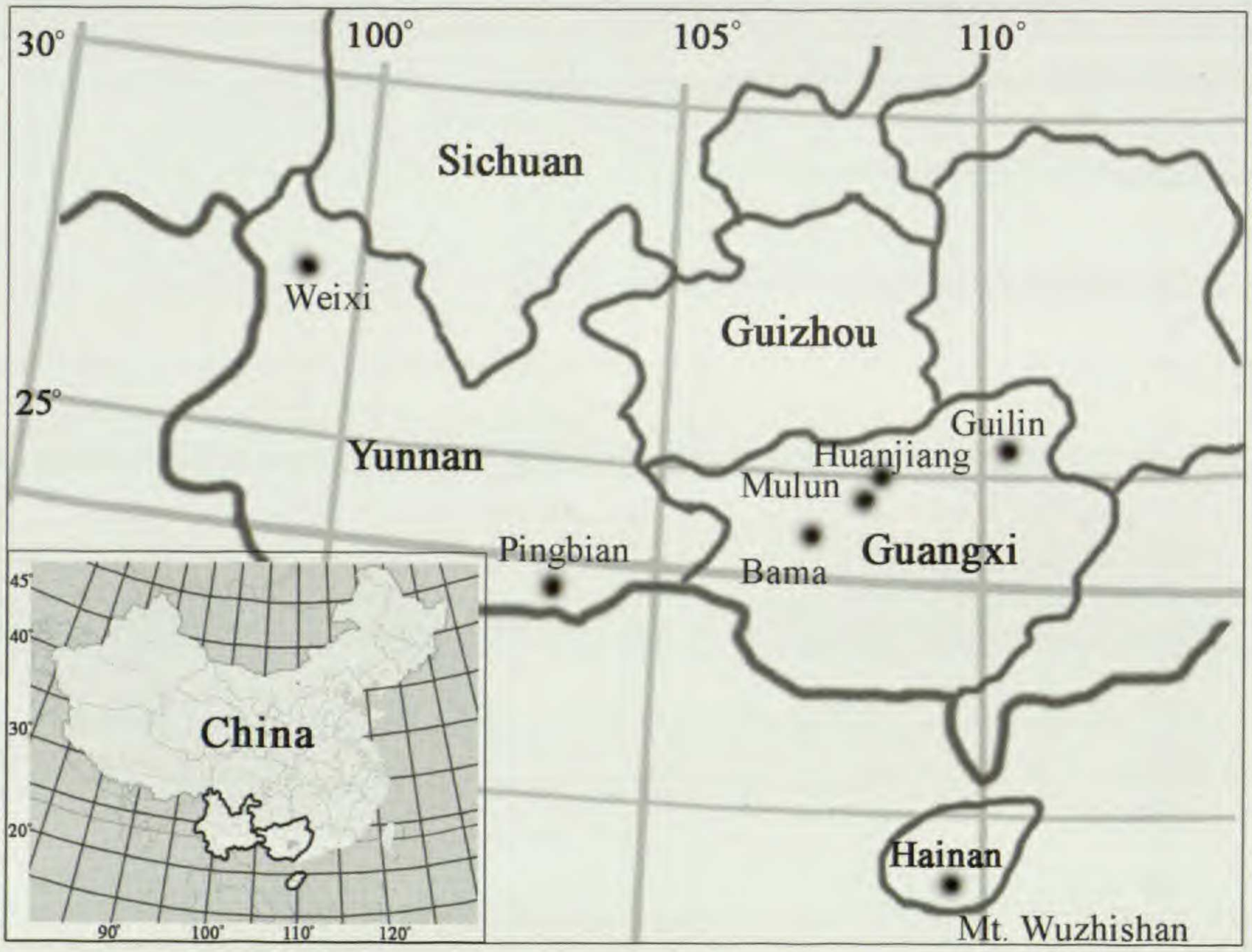

FIG. 1. Distribution map showing localities of the seven collection sites.

type with 32 spores (Manton, 1950; Lovis, 1977; Walker, 1979). To determine the reproductive type, the number of spores per sporangium was counted. At least five unopened sporangia were counted in each individual.

RESULTS AND DISCUSSION

The chromosome photographs of root tip materials of 12 species in eight genera of Polypodiaceae are shown in Figures 2 and 3. The chromosome numbers for four species are newly reported for China: $2n=72$ ($2x$) in *Lepidogrammitis drymoglossoides*, $2n=72$ ($2x$) in *Neolepisorus ovatus*, $2n=72$ ($2x$) in *Phymatopteris rhynchophylla*, and $2n=72$ ($2x$) in *Phymatosorus hainanensis*. Four records are new cytotypes: $2n=72$ ($2x$) in *Colysis hemionitidea*, $2n=72$ ($2x$) in *Lepisorus thunbergianus*, $2n=108$ ($3x$) in *Phymatopteris crenatopinnata*, and $2n=108$ ($3x$) in *Phymatosorus cuspidatus*. *Lepisorus thunbergianus* $2n=72$ ($2x$) has the base number $x=36$, which is different from the previous reports $x=25$, 38, 50, and 51. We confirmed the counts of $2n=72$ ($2x$) in *Colysis elliptica*, $2n=72$ ($2x$) in *C. pothifolia*, $2n=72$ ($2x$) in *Microsorium fortunei*, and $2n=72$ ($2x$) in *Pyrrosia lingua*.

The reproductive type in *P. cuspidatus* and *P. crenatopinnata* was apogamous, whereas in the other species it was of the sexual reproductive type. The chromosome number and reproductive mode for each species are described and discussed below.

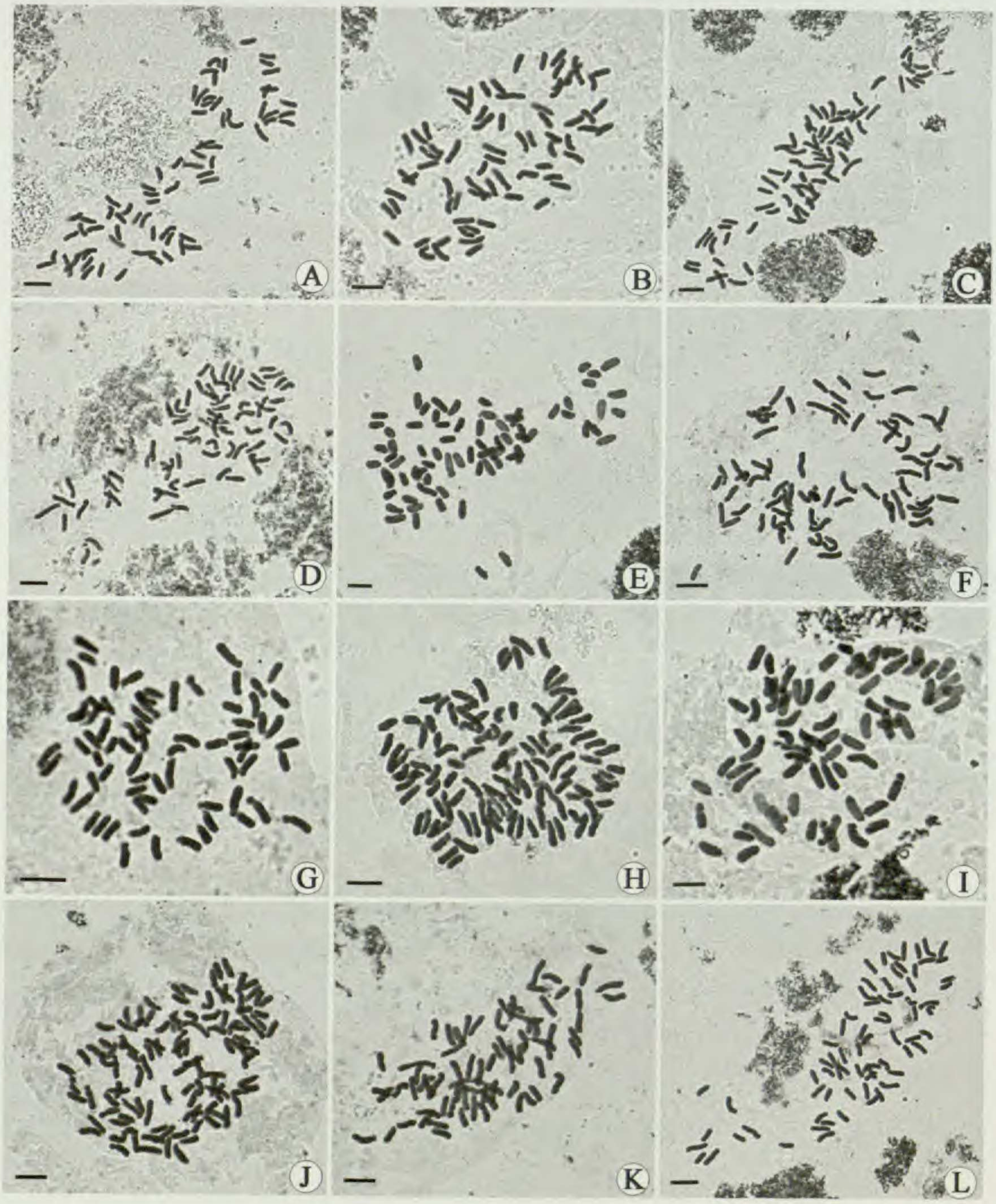

FIG. 2. Photographs of somatic chromosomes at metaphase A. *Colysis elliptica*, 2*n*=72. B. *C. hemionitidea*, 2*n*=72. C. *C. pothifolia*, 2*n*=72. D. *Lepidogrammitis drymoglossoides*, 2*n*=72. E. *Lepisorus thunbergianus*, 2*n*=72. F. *Microsorium fortunei*, 2*n*=72. G. *Neolepisorus ovatus*, 2*n*=72. H. *Phymatopteris crenatopinnata*, 2*n*=108. I. *P. rhynchophylla*, 2*n*=72. J. *Phymatosorus cuspidatus*, 2*n*=108. K. *P. hainanensis*, 2*n*=72. L. *Pyrrosia lingua*, 2*n*=72 (*scale bars*=5μm).

***Colysis elliptica* (Thunb.) Ching**

This species is known from across India, Vietnam, Japan, and southern China. The material from Mulun, Guangxi counted in this study had 72 chromosomes in the mitotic cells (Fig. 2A, 3A). The species is a diploid, with 64 spores per sporangium.

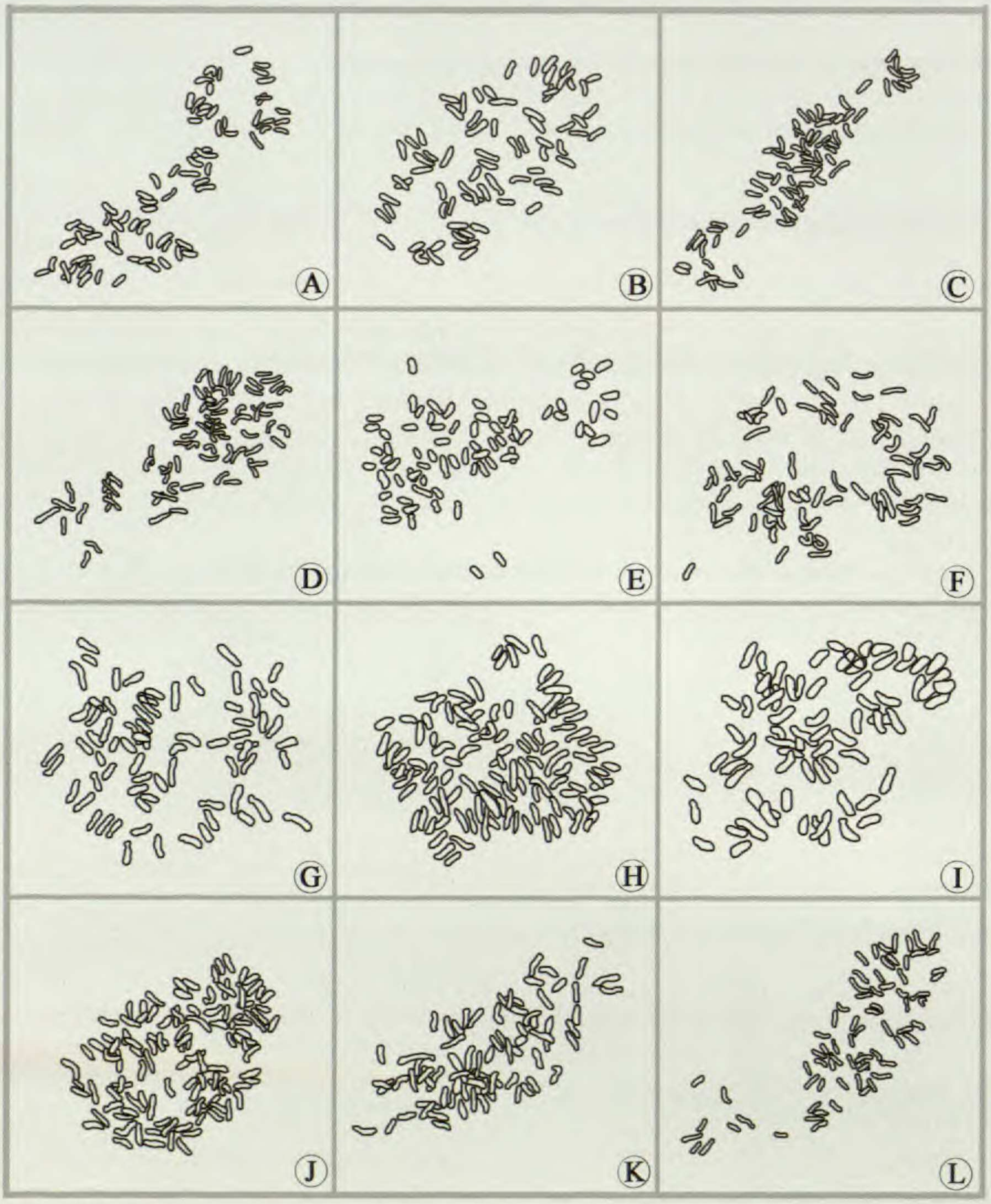

FIG. 3. Explanatory drawings to Fig. 2. A. *Colysis elliptica*, 2*n*=72. B. *C. hemionitidea*, 2*n*=72. C. *C. pothifolia*, 2*n*=72. D. *Lepidogrammitis drymoglossoides*, 2*n*=72. E. *Lepisorus thunbergianus*, 2*n*=72. F. *Microsorium fortunei*, 2*n*=72. G. *Neolepisorus ovatus*, 2*n*=72. H. *Phymatopteris crenatopinnata*, 2*n*=108. I. *P. rhynchophylla*, 2*n*=72. J. *Phymatosorus cuspidatus*, 2*n*=108. K. *P. hainanensis*, 2*n*=72. L. *Pyrrosia lingua*, 2*n*=72.

Tsai and Shieh (1983) reported the material from Taiwan, China had 36 chromosomes in the meiotic cells. Weng (1990) suggested the material from Fujian, China also had 36 chromosomes in the meiotic cells, and 64 spores per sporangium, which suggests it is a diploid. Kato *et al.* (1992) believed the material from Dali, Yunnan, had 72 chromosomes in the mitotic cells. The

materials from five Japanese populations had 36 chromosomes in the meiotic cells (Mitui, 1966, 1968; Kurita, 1968; Takei, 1983b), and 64 spores per sporangium, which suggested that these species are sexual diploids. These results indicated stability of chromosome numbers in the different populations in China and Japan.

The record made by Löve *et al.* (1977) showed that different chromosome numbers occur in six species of the genus *Colysis*, indicating that diploid ($2n=72$), triploid ($2n=108$), tetraploid ($2n=144$), and hexaploid ($2n=216$) counts exist in this genus.

***C. hemionitidea* (Wall. ex Mett.) C. Presl**

This species is widespread in tropical and subtropical Asia. The material from Guilin, Guangxi counted in this study had 72 chromosomes in the mitotic cells (Fig. 2B, 3B). Sixty-four spores per sporangium were found, suggesting it is a sexual diploid. This record is a new cytotype.

Mehra (1961) shown the material from Mt. Himalaya had 144 chromosomes in the mitotic cells, and 64 spores per sporangium, suggesting it is a sexual tetraploid.

***C. pothifolia* (D. Don.) C. Presl**

This species is widespread in tropical and subtropical Asia. The material from Guilin, Guangxi counted in this study had 72 chromosomes in the mitotic cells (Fig. 2C, 3C). We suggest this species is a sexual diploid based on the observation of 64 spores per sporangium.

Tsai and Shieh (1983) found that the material from Taiwan, China had 36 chromosomes in the meiotic cells. Kato *et al.* (1992) collected material from Dali, Yunnan and Lin *et al.* (2002) collected materials from Fujian, each of which had 72 chromosomes in the mitotic cells. All materials above had 64 spores per sporangium, suggesting that these are sexual diploids.

***Lepidogrammitis drymoglossoides* (Bak.) Ching**

This species is endemic to China, distributed widely in the southern region. The material from Bama, Guangxi counted in this study had 72 chromosomes in the mitotic cells (Fig. 2D, 3D). Sixty-four spores were present in each sporangium, suggesting that the species is a sexual diploid. This chromosome count is reported for the first time.

***Lepisorus thunbergianus* (Kaulf.) Ching**

This species is known from across southern China, Japan, and the Philippines. The material from Huanjiang, Guangxi in this study was found to have $2n=72$ chromosomes in the mitotic cells (Fig. 2E, 3E). The species is a sexual diploid, with 64 spores per sporangium. This is a new cytotype.

Lin *et al.* (2002) reported that the material from Fujian had $2n=100$ chromosomes in the mitotic cells, and suggested it was a sexual tetraploid.

Lepisorus thunbergianus is a cytologically complicated species, its chromosome numbers have been variously reported as $n=25$, 50, $2n=50$, 51, 75, 76, 100, 101, 102 and 103, including examples of polyploidy and aneuploidy from Japan (Takamiya, 1996). It has been shown that diploids, triploids, tetraploids, and polyploids exist in *L. thunbergianus* (Takamiya, 1996).

***Microsorium fortunei* (T. Moore) Ching**

This species is known from across southern China, Burma, Bhutan, and Vietnam. Material from Guilin, Guangxi in this study had 72 chromosomes in

the mitotic cells (Fig. 2F, 3F). Sixty-four spores were present in each sporangium, suggesting it is a sexual diploid.

Roy and Holttum (1965) reported *M. fortunei* from southern China had 36 chromosomes in the meiotic cells, suggesting the same results as this paper. In contrast, Mitui (1973) suggested the material from Japan had 37 chromosomes in the meiotic cells, which may be the result of aneuploidy.

***Neolepisorus ovatus* (Bedd.) Ching**

This species is widespread in southern regions of China and India. The material from Guilin, Guangxi counted in this study had 72 chromosomes in the mitotic cells (Fig. 2G, 3G). There were 64 spores per sporangium. The results suggest it should be considered a sexual diploid species. This chromosome count is reported for the first time.

***Phymatopteris crenatopinnata* (C. B. Clarke) Pichi-Serm.**

This species occurs in southern China and also in NE India. The material from Weixi, Yunnan, had 108 chromosomes in the mitotic cells (Fig. 2H, 3H). We counted 32 spores per sporangium. The results suggest it is apogamous with $2n=108$ in this study. This is a new cytotype.

Panigrahi and Patnaik (1961) reported the material from eastern India had 72 chromosomes in the mitotic cells, and 64 spores per sporangium, suggesting it is a sexual diploid.

***P. rhynchophylla* (Hook.) Pichi-Serm.**

This species is widespread in tropical and subtropical Asia. The material we collected from Pingbian, Yunnan, had 72 chromosomes in the mitotic cells (Fig. 2I, 3I). There were 64 spores per sporangium, suggesting it is a sexual diploid. This chromosome count is reported for the first time.

***Phymatosorus cuspidatus* (D. Don.) Pichi-Serm.**

This species is known from across southern China, India, Vietnam, Nepal, and Thailand. The material from Bama, Guangxi counted in this study had 108 chromosomes in the mitotic cells (Fig. 2J, 3J). Thirty-two spores were present per sporangium. The results indicate it is an apogamous triploid with $2n=108$ and it represents a new cytotype.

Kato and Nakato (1999) reported the material from Hainan had 74 chromosomes in the mitotic cells, and 64 spores per sporangium, suggesting it is a sexual diploid.

***P. hainanensis* (Noot.) S. G. Lu**

This species is found in Hainan, China and also in India and Vietnam. The material we collected from Mt. Wuzhishan, Hainan, had 72 chromosomes in the mitotic cells (Fig. 2K, 3K). We counted 64 spores per sporangium, suggesting it is a sexual diploid. This chromosome count is reported for the first time.

***Pyrrosia lingua* (Thunb.) Farwell**

This species is known from across southern China, India, Vietnam, and Japan. The material from Pingbian, Yunnan counted in this study had 72 chromosomes in the mitotic cells (Fig. 2L, 3L). The species is a sexual diploid, with 64 spores per sporangium.

Takei (1969, 1983a) believed the material from Japan also had 72 chromosomes in the mitotic cells. Our result suggests that *Pyrrosia lingua* is in a stable state.

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